undergo surface induced osteogenesis. We put forward a mechanotransductive model to interpret these and other's findings.

3578-Pos Board B439
Mesenchymal Stem Cells Duro- and Hapto- Taxis Alters Cell State
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Anchorage dependent cells can sense and respond to extracellular matrix (ECM) stiffness, but stiffness gradients are often found in vivo via normal tissue variation or pathological conditions, such as the post-infarct myocardial scar which is several folds stiffer than healthy tissue. We have previously shown that mesenchymal stem cell (MSC) differentiation as well as migration is regulated by substrate stiffness in 2D in vitro; it is important to determine if this also occurs in 3D as it could explain MSC migration and calcification in infarct scars in vivo. 3 mg/ml collagen hydrogels were crosslinked with 1, 5, 10, and 20 mM genipin, a natural nontoxic collagen crosslinker, for 2, 4, 12, 24, and 48 hours to yield physiological stiffnesses ranging from 0.9 to 6.4 kPa. MSC behavior on these matrices mimicked previous reports. 3D gradient hydrogels with encapsulated MSCs, fabricated using microfluidics to diffuse genipin through a cell-collagen network, should result in a spatial gradient of crosslinking across the gel. MSCs proliferation, migration, morphology, and differentiation in the presence of this 3D gradient will be compared with 2D gradients in which MSCs were observed to first migrate and then differentiate. MSC durotaxis during wound healing in vivo may be additionally accompanied by haptotaxis, migration due to a matrix ligand gradient, as a result of localized matrix secretion by fibroblasts at the site of regeneration. 2D haptotatic hydrogels with gradients of collagen and fibronectin were also made and can be overlayed onto stiffness gradient hydrogels in order to investigate the effect of corresponding and opposing gradients on MSC behavior. These data show that MSC migration and subsequent differentiation can be regulated by a variety of ECM stimuli in addition to growth factor-mediated pathways.

3579-Pos Board B440
Natural Killer Cell-Mediated Tumor Surveillance: Correlation Between Killing Efficiency, Transient Migration Behavior and Morphology
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We recently developed a simple method for automatic characterization of transient migration behavior of natural killer (NK) cells imaged by fluorescence time-lapse microscopy [1]. For imaging we used a microchip-based assay where small numbers of NK and tumor target cells were confined inside microwells. Single-cell tracking revealed differences between
NK cells as well as transient changes in migration behavior for individual NK cells. Frequently, NK cells were found to have periods of high motility, interrupted by periods of slow migration or complete stops. Such transient migration arrest periods (TMAPs) often coincided with formation of immune synapses with target cells, but NK cells were also observed to stop spontaneous and prior to cell division or death. To objectively characterize NK cell migration, we used a sliding window approach to evaluate local migration coefficients and mean squared displacement curvature in individual cell trajectories. Three modes of migration were distinguished: TMAPs, directed migration and random movement. Here, we correlated transient migration behavior with NK cell morphology. In general, NK cells were more circular during immune synapse formation and in TMAPs compared to when in directed migration or random movement. On the other hand, motile NK cells had bigger areas and perimeters compared to NK cells engaged in immune synapses or in TMAPs. The method presented can quickly and quantitatively assess migration and morphological properties of different cell populations. Current analysis is focused on determining whether particularly cytotoxic NK cells (serial killers) display distinct properties from other subpopulations of NK cells.
1. Khorshidi, M.A., et al., *Analysis of transient migration behavior of natural killer cells imaged in situ and in vitro.* Integr Biol (Camb), 2011. **3**(7): p. 770-8.

3580-Pos Board B441
Cancer Migration in 3-D Environment
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Cell migration plays an important role in various biological processes ranging from tissue repair to cancer metastasis. Most of the studies reported so far have investigated cell migration in a 2-D environment. However, recent years have witnessed a surge of interest in understanding migration of cells within 3-D environment recapitulating various attributes of the native environment. To this end, we have developed an approach to study the migratory transition from 2-D to 3-D on Matrigel as a model system to mimic the processes, including matrix degradation, occurring in 3-D migration. Using this system, we characterize the migration capabilities of non-metastatic (MCF-7) and metastatic (MDA-MB-231) breast cancer cells by analyzing (i) the penetration depth into the Matrigel required for plastic deformation of the matrix, (ii) the magnitude and distribution of traction forces generated before the elastic limit of the material. Furthermore, we examine whether the cellular motility between non-metastatic and metastatic cells converge to a similar behavior when the matrix metalloproteinase activity are altered at various levels. The degree of convergence will be determined by again analyzing the required depth for plastic deformation and the magnitude and distribution of traction forces generated before the elastic limit.

3581-Pos Board B442
Differential Effects of Serum Heat Treatment on Chemotaxis and Phagocytosis by Human Neutrophils
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The ability of certain white blood cells to actively change their shape is not only a cornerstone of a host's innate immune defense, but also provides a highly instructive, cross-disciplinary window into eukaryotic cell motility. Recent findings have indicated that human neutrophils rely at least partially on similar biophysical mechanisms during chemotaxis and phagocytosis. To further elucidate similarities and differences between these two prominent examples of immune-cell motility, we here examine the effects of heat treatment of serum components on neutrophil interactions with antibody-coated beads and zymosan particles. Because heat treatment can inhibit some or all viral activity in the serum, it is a common method to protect laboratory personnel, and also has many other applications. Here, we incubate neutrophils with the two target types in buffers containing serum that had been treated at a range of different temperatures. To discriminate between phagocytic and chemotactic behavior, we quantify the cell response by combining flow cytometry bulk assays with single-cell/single-target experiments based on dual-micropipette manipulation. We find that heat treatment of serum at 52°C or higher significantly depressed phagocytosis of opsonized zymosan in our bulk assays for all donors tested. On the other hand, single-cell experiments demonstrated complete inhibition of chemotactic activity already at 48°C, even though the neutrophils still engulfed target particles that were brought into physical contact with the cells using micropipettes. This implies that, although chemotactic stimulation of neutrophils is not required for phagocytosis, it can significantly enhance the phagocytic response. Our results also demonstrate that by fine-tuning heat treatment of serum, one can selectively study chemotaxis or phagocytosis under otherwise identical conditions.

3582-Pos Board B443
Tangential Tether Extraction and Spontaneous Tether Retraction of Human Neutrophils
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Membrane tethers are extracted when neutrophils roll on the endothelium to initiate their transendothelial migration. Tether extraction from both neutrophils and endothelial cells stabilizes neutrophil rolling, so it has been studied extensively and the force-velocity relationship for tether extraction is of great interest. Limited by the techniques in previous studies, this relationship has been only obtained from tethers perpendicular to the cell surface. Here, with the micro-cantilever technique where latex beads affixed on silicon cantilevers were used as the force transducer, we extracted tethers either perpendicular or tangential to the neutrophil surface. We found that the force-velocity relationship was not sensitive to tether pulling direction. Little movement of the tether-cell junction was observed during tangential tether extraction and no coalescence was observed during multiple tether extraction. Following adhesion rupture, spontaneous tether retraction was visualized by membrane staining, which revealed two phases: one was fast and exponential, whereas the other was slow and linear. Our mechanistic modeling findings showed some similar tether behaviors. Therefore, these results illustrate for the first time how neutrophil tethers shorten upon instantaneous force removal and illustrate further how membrane tethers contribute to neutrophil rolling stability during the inflammatory response.